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The Potential Use of Raman Mapping To Investigate In Vitro Deposition of Combination Pressurized Metered-Dose Inhalers

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ABSTRACT

Scanning near-infrared Raman microscopy has been used to map aerosol particulate deposits produced from pressurized metered-dose inhalers (pMDI). A commercially available combination asthma therapy pMDI (Ventide, Allen and Hanbury, UK), containing salbutamol and beclometasone dipropionate, was analyzed by conventional in vitro quantitative analysis and scanning Raman microscopy. Raman maps, taken from Andersen cascade impactor plate stages 3 and 5 (over 100 × 100 μm areas) suggested good correlation with chemical analysis of the respective stages. Scanning Raman microscopy allows visual differentiation between formulation components (not possible using conventional imaging techniques), while potentially allowing chemical quantification.

KEYWORDS: pMDIs, combination therapy, Raman mapping, SEM, Andersen Cascade Impactor.

INTRODUCTION

Standard analysis of pharmaceutical formulations uses a series of in vitro and physical characterization tools. For pressurized metered dose inhalers (pMDI), these techniques include electron microscopy, chemical analysis, and the determination of aerosol particle size distribution.

The Andersen Cascade Impactor (ACI) (Copley Scientific, Nottingham, UK) is a standard in vitro technique used to obtain particle size distribution information from aerosol plumes for pulmonary delivery. Aerosol particles are classified into well-characterized aerodynamic diameter ranges from which the regional lung deposition can be assigned. Analysis of the deposition profiles may highlight formulation stability issues such as particle adhesion to device components, agglomeration of actives, and physico-mechanical stability. However, the analytical techniques commonly used provide limited information pertaining to such physical interactions. Chemical mapping has the potential to yield this information.

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Spatial resolution for Raman mapping of 1-2 μm can routinely be achieved without deconvolution of the data (in comparison to 10 μm for mid-infrared [mid-IR][1] and therefore has the potential for the detailed, rapid, and routine analysis of pharmaceutical materials.

Previously, Raman spectroscopy has been used to demonstrate the presence of drug-drug or drug-excipient interactions[2] and in the mapping of drugs in solid dispersions[3] and transdermal patches.[4] However, to date, Raman mapping has not been used in investigations involving pMDIs or dry powder inhalers (DPIs).

MATERIALS AND METHODS

Materials

Salbutamol, beclometasone dipropionate (BDP) (both *British Pharmacopoeia (BP)* grade), and oleic acid (*BP/ European Pharmacopoeia (EP)* grade, Oleotec Ltd, Cheshire, UK) were used as high-performance liquid chromatography (HPLC) and Raman standards. The commercial combination therapy pMDI used was Ventide (Allen and Hanbury, Herts, UK; salbutamol 100 μg, BDP 50 μg, batch number D025809). An unspecified concentration of oleic acid was present in the formulation as a cosolvent. The HPLC solvent used throughout was 50/50 v/v acetonitrile (far UV HPLC grade, Fisher, Loughborough, UK) and water (MilliQ purified, Millipore, Watford, UK).

In Vitro Aerosol Deposition Profiles

Aerosol deposition patterns from the Ventide combination product were analyzed by an ACI fitted with a metal throat and pMDI mouthpiece adaptor (Apparatus 2, *EP*, 1999). The pMDI cans were shaken thoroughly between each actuation. Five actuations to waste were conducted with a dummy actuator prior to analysis, to prime the metering valve. Ten shots were discharged to the ACI at a flow rate of 28.3 $Lmin^{-1}$ (±5%) under conditions specified in the *BP*. Shot weights were recorded between actuations (can and actuator weight) with a 60-second lag between measurements.

Drug deposits were recovered by rinsing each ACI stage plate into suitable volumetric flasks with HPLC mobile phase before adjusting to volume. The throat, mouthpiece-

Table 1. Summary of the BDP and Salbutamol Sulfate Drug Deposition in the ACI*

	BDP	Salbutamol Sulfate
Emitted dose (μg)	45.3 ± 1.4	119.6 ± 2.4
Delivered dose (μg)	37.7 ± 2.9	100.5 ± 6.6
Fine particle dose (μg)	13.8 ± 1.2	48.3 ± 3.3
MMAD (μm)	3.7 ± 0.2	2.4 ± 0.2
GSD	1.4	1.8

*BDP indicates beclometasone dipropionate; ACI, Andersen Cascade Impactor; emitted dose, total recovery; delivered dose, theoretical amount to patient (less actuator); fine particle dose, stage 3 to filter (particles with a mass mean aerosol diameter [MMAD] of less than 4.7 μm); and GSD (Geometric Standard Deviation). Results are represented as individual dose (ie, division by 10) (n = 3).

adapter, and pMDI actuator were rinsed separately using mobile phase and adjusted to volume in suitable volumetric flasks. In addition, the filter stage was recovered by sonication in mobile phase, filtration through a 0.2-μm polytetrafluoroethylene (PTFE) filter (Whatman Inc, Clifton, NJ), and volume adjustment. The aerosol distribution functions, obtained from recovered drug concentrations, were calculated by the CITDAS software package (Copley Scientific Ltd, Nottingham, UK). All ACI studies were run in triplicate using 3 Ventide pMDIs from the same batch. A validated HPLC method was used for the quantitative analysis of the washings collected from the ACI, after appropriate dilution where necessary.

Scanning Electron Microscopy

Scanning electron photomicrographs were collected using a T330 (Jeol Ltd, Herts, UK) scanning electron microscope (SEM). Images were collected using an accelerating voltage of 10 kV over the magnification range ×100 to ×5000 original magnification. The plates were sputter coated with gold (Edwards Sputter Coater, BOC Edwards, Sussex, UK) prior to analysis. By referencing the markings on each steel disc, the deposit analyzed in the Raman experiments could be imaged.

Raman Mapping

Samples of BDP, salbutamol sulfate, salbutamol base, and oleic acid were analyzed to obtain their Raman spectra. These spectra were subsequently used as references to identify the components on the mapped samples. Deposits from stage 3 and stage 5 (size ranges 3.3-4.7 μm and 1.1-2.1 μm for stage 3 and 5, respectively) of the ACI using a single actuation of the Ventide inhaler were collected for Raman mapping. Deposits were collected using the method of Clarke et al,[5] wherein 1 cm diameter steel discs were placed at the edge of the inverted ACI plates so as to maintain the jet to plate gap. In addition, an asymmetrical marking was scratched onto the surface of the disc so that deposits could be identified.

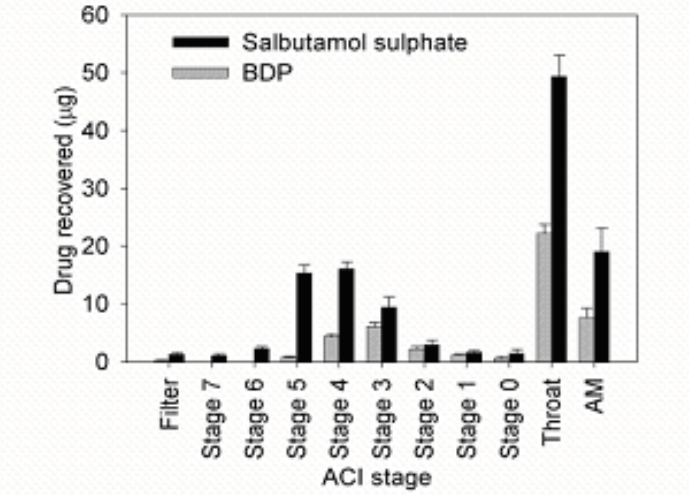

Figure 1. Histogram of results from the chemical analysis of the deposits from the Andersen Cascade Impactor.

Raman spectra were collected using an RM1000 (G97005) Raman system (Renishaw, Gloucestershire, UK). Excitation was achieved using a 785-nm high-performance near-IR (HPNIR) diode laser (Renishaw), focused through a ×50 objective (N PLAN L long working distance, Leica, Milton Keynes, UK) and filtered to give ~10 mW total laser energy at the sample. Spectra were collected in static mode over the range 1338 cm^{-1} to 1829 cm^{-1} with a 30-second scan time. Data for Raman maps were collected over a 100 × 100 μm area using a step size of 1 μm, controlled by associated software. The intensity of Raman bands at 1610 cm^{-1} (salbutamol) and 1662 cm^{-1} (BDP) were mapped to investigate the relative amount of each component. A ratio of these peak intensities was performed (1610/1662) with an arbitrary value of 100 counts being added artificially to each point to remove ratio problems associated with noise where no active component is present. The Raman bands at 1610 cm^{-1} and 1662 cm^{-1} were chosen because minimal interference was observed from the other formulation components.

RESULTS

In Vitro Aerosol Deposition Profiles

The in vitro ACI deposition of salbutamol and BDP determined from 10 actuations of the Ventide inhaler are summarized in Table 1. Figure 1 shows the relative deposition masses for salbutamol and BDP on the ACI stages. A mean shot weight of 83.2 ± 1.6 mg was recorded for the 30 actuations.

Scanning Electron Microscopy

A representative electron photomicrograph from Plate 3 is reproduced in Figure 2. The image shown is the same deposit and the area is of a similar size to that analyzed by Raman mapping, but with a different orientation. In general, irregu-

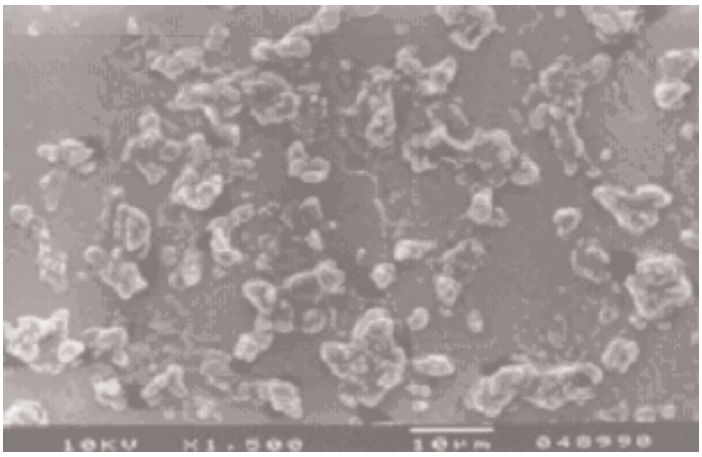

Figure 2. Representative SEM of the deposits recovered from stage 5 of the Andersen Cascade Impactor. Although the morphology of the deposits can be clearly seen, it is not possible to distinguish between the drug components.

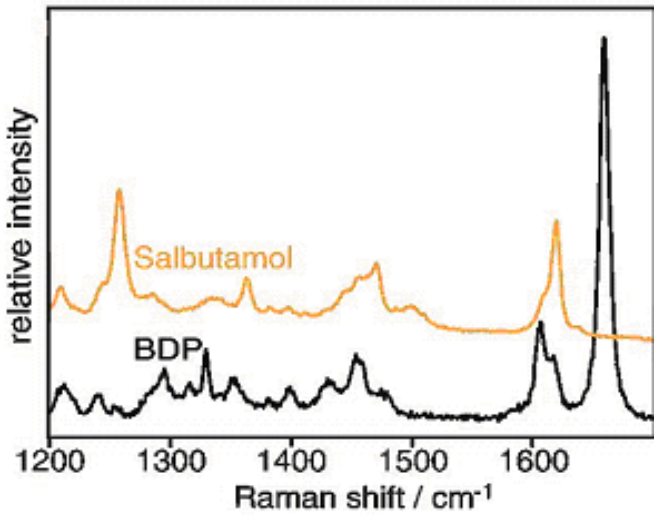

Figure 3. Raman spectra of pure samples of the active components in the Ventide formulation. Wavenumber range 1300 to 1800 cm^{-1}.

lar particle morphology was evident. As expected, no differentiation between active components was possible.

Raman Mapping

Reference spectra collected for BDP and salbutamol are compared in Figure 3. Raman maps (taken over 100 × 100 mm areas) for stages 3 and 5 ACI deposits are shown in Figure 4, A and B, respectively. Data are presented as an orange intensity ratio map where orange regions indicate salbutamol.

DISCUSSION

In Vitro Aerosol Deposition Profiles

From the data in Table 1 and the histogram in Figure 1, it can be seen that the majority of drug deposition occurs on the

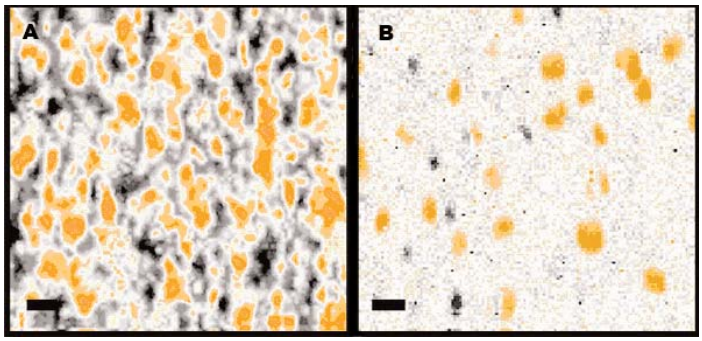

Figure 4. Raman intensity ratio map of deposit from stages 3 (left) and 5 (right) of the ACI. Ratio of intensity of 1610 cm^{-1} (salbutamol), and 1662 cm^{-1} (BDP). Data have been treated so that orange (light) components relate to salbutamol and black components relate to BDP. Scale bars represent 10 µm.

ACI throat and actuator. The delivered dose of BDP is less than half that of salbutamol, reflecting the stipulated values of 50 µg and 100 µg. However, it is important to note that the delivered dose for BDP is lower than the stated dose, a variability that may be expected from a dosage form held in suspension.

Salbutamol has a smaller mean particle size than BDP. This is reflected by fine particle doses and corresponding fine particle fractions of 45.2% ± 2.4% and 30.7% ± 2.35% for salbutamol and BDP, respectively.

Stages 3 and 5 were chosen because the relative distributions of the 2 drugs were independent. Stage 3 concentrations were 6.1 ± 0.7 µg and 9.4 ± 1.9 µg for BDP and salbutamol, respectively. Stage 5 concentrations were 0.8 ± 0.2 µg and 15.4 ± 1.4 µg for BDP and salbutamol, respectively.

Raman Mapping

Raman mapping yields information additional to that obtained from SEM images; in Figure 2 it is not possible to distinguish between the drug components on the basis of deposit morphology. On the plate with the lower drug concentration (Plate 3), an approximate correspondence between the particle size range stated for the ACI stage under investigation and the size of the deposits can be observed. This cannot be done for the higher dose drug on the plate, since the accumulation of individual particles appears to yield large agglomerates.

The mass balance and the HPLC analyses show that there is no detectable decrease in the bioavailabilty of either drug, with no significant decrease in the total delivered doses. This finding suggests that no sample degradation or drug-drug or drug-excipient interactions have occurred. This can be confirmed using the Raman spectra of the deposits, where analysis of the variability of the spectra of the components shows no significant change in the Raman shift of the major peaks for each component or the presence of additional peaks, indicative of new bond formation. An interesting point is that

the BDP in the Ventide formulation is believed to be in the anhydrous state. In the presence of moisture, the anhydrous state converts to the monohydrate. Such transformation may take place via water penetration into the canister or postactuation. Although no difference between the peaks of the BDP standard and ACI deposits were observed, such studies are worthy of future investigation. In addition, no oleic acid could be detected in the spectra. Oleic acid may be present as a thin film at a concentration below the detection limits of the technique.

Image analysis of the maps shows some potential as a means to obtain quantitative data from the Raman maps. Intensity maps were processed through a threshold transform and exported to produce a data set (including particle type, dimension, and area). In general, the total area ratio for both salbutamol and BDP were in good agreement with the chemical analysis of stage 3 deposits, with a poorer correlation for stage 5 deposits. The ratios of the total areas ascribed to the 2 drugs on stages 3 and 5 were 1.3 and 5.2, respectively. This compares with ratios of 1.1 and 20.3 for the HPLC analyses of the drug deposits on stages 3 and 5, respectively. No calibration was undertaken for this study, hence only a qualitative relationship can be established. The linear range of such a method will be restricted at higher concentrations, since the formation of mounds of significant thickness may reduce the usefulness of a strictly 2-dimensional scanning technique. This will be the principal reason for the poor correlation between the ACI and image analysis results in stage 5.

Conclusion

Raman mapping in conjunction with standard in vitro techniques may provide useful information relating to physicochemical and physicomechanical properties of pMDI formulations. In addition, image analysis showed that there is a potential for quantitative analysis of the deposits, although careful calibration will be required.

However, the real power of scanning Raman spectroscopy is the potential for direct chemical visualization and differentiation of materials, thus making it applicable to all solid dosage forms, including pMDIs, tablets, and DPI technology. In the future, it would be interesting to investigate deposition patterns from multicomponent DPI systems and the influence of humidity on the stability and/or polymorphic forms of pharmaceutical components.

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